Mesenchymal Stem Cell Secretome Ameliorates Hyperalgesia in a Post-operative Pain Model

Chrysostomos Constantine Maoudis (chrys.maoudis@regeneus.com.au)
Regeneus Ltd; University of New South Wales https://orcid.org/0000-0002-7376-5050

Flyn McKinnirey
Regeneus Ltd

Graham Vesey
Regeneus Ltd

Sinead Blaber
Regeneus Ltd

Mark Hutchinson
The University of Adelaide; ARC Centre of Excellence for Nanoscale BioPhotonics

---

Research

Keywords: Mesenchymal Stem Cells (MSC), secretome, pain, post-operative pain, hotplate, Von Frey

Posted Date: July 19th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-709385/v1

Abstract

Background

The treatment and management of post-operative pain remain a significant challenge. There is a significant unmet need that requires novel therapeutics. The aim of this study was to investigate the effects of the mesenchymal stem cell secretome (MSC-S) in a rat incisional post-operative pain model.

Methods

A post-operative pain model in rats was used to assess the human adipose-derived MSC-S therapeutic benefits. Male Sprague Dawley (SD) rats (n=40) underwent surgery whereby a 1 cm longitudinal incision was made over the plantar surface of the right hind paw where the plantaris muscle was incised longitudinally. The incision was closed with two stitches. At 1-hour post-surgery, test and control articles were administered topically for 15 minutes to the incision site. Von Frey and hotplate testing were conducted to assess mechanical and thermal pain responses, respectively. Statistical analysis of thermal and mechanical withdrawal thresholds was undertaken using an ordinary 2-way analysis of variance (ANOVA) utilising uncorrected Fishers LSD test with individual variances computed for each comparison.

Results

A statistically significant increase in thermal nociceptive threshold (analgesia) was observed in the high dose MSC-S group when compared to vehicle at 1.5 hours ($p<0.0001$) and 3 hours post-surgery ($p<0.0001$). High dose MSC-S had a more potent analgesic effect than low dose MSC-S. A statistically higher analgesic effect was observed in the high dose MSC-S group compared to morphine at the 3 hours post-surgery hot plate assessment ($p<0.002$). Morphine (10mg/kg) administration resulted in a significant increase in hot plate withdrawal threshold compared to vehicle at 1.5 hours ($p<0.0001$) and 3hours ($p<0.008$) post-surgery. Animals that received morphine displayed significantly less allodynia than vehicle control animals at 1.5 hours post-surgery ($p<0.0001$).

Conclusions

The present study demonstrated that the MSC-S topically administered in a rat post-operative pain model significantly improved thermal nociceptive thresholds. The MSC-S has potential as a non-opioid based therapy for the treatment of acute post-operative pain.

Background

The prevalence of post-operative pain varies from 10-80% in patients following surgery (1–4). Acute post-operative pain is caused by post-surgical trauma resulting in an inflammatory reaction and hyperalgesia (5,6). Hyperalgesia at the region of the surgical trauma is mediated by sensitisation of the peripheral afferents and hyperexcitability of A-Delta and C-fibre nociceptors (5).

Although post-operative pain is a phenomenon normally described in acute settings, recent studies have demonstrated that poorly managed post-operative pain can lead to chronic pain (1). In this scenario, chronic pain can develop via central sensitisation, where higher brain centre systems play a role in the development and persistence of post-operative pain long after the trauma has healed (5).

Numerous biopsychosocial factors can contribute to acute post-operative pain (7). Various interventions are currently utilised to mitigate the factors that exacerbate exaggerated pain states and achieve effective post-operative pain management, including access to acute management teams, patient education, balanced use of analgesic medication, and regular pain assessments to enable personalised care (7).

Currently, acute post-operative pain management involves initial opioid analgesic administration. However, physicians aim to minimise the duration of opioid use to avoid common adverse events associated with opioid use (8). These strategies have resulted in decreased patient morbidity and an increase in patients' physical and mental state post-surgery, thereby reducing the risk of chronic pain development (5,9). Nevertheless, there is a need for more effective post-operative pain treatments. This is because despite the improvements in post operative pain management 50% percent of patients remain sub-optimally treated(10). Ineffective post-operative pain management leads to increased morbidity, decreased quality of life, delayed recovery, prolonged opioid use, and higher healthcare costs (4). Effective treatment of acute post-operative pain can reduce psychological stress contributing to central sensitisation seen in chronic post-operative pain patients (5). Therefore, an alternative, safe, preferably non-opioid analgesic treatment is urgently needed to address this significant unmet need.

Adipose-derived mesenchymal stem cells (AdMSCs) are multipotent adult stem cells with significant therapeutic potential in various indications. It is now accepted that MSCs do not exert their therapeutic effect via their differentiation potential but rather via direct cell-cell contact and through paracrine effects of their bioactive secretome (11). These MSC-S contain a myriad of bioactive substances, many of which have been examined for their antinociceptive and antiallodynic qualities in isolation and are hypothesised to contribute to the MSCs reversal and prevention of the induction of neuropathic pain mechanical allodynia and thermal hyperalgesia (12–40). However, further investigation is warranted to understand the MSCs mechanism of action via their MSC-S factors. Therefore, we aimed to conduct the first study to investigate the benefits of the adipose-derived MSC-S in a rat incisional acute post-operative pain model, with a benchmarking of the analgesic effect to a robust dose of subcutaneous morphine. We hypothesised that topical application of MSC-S in the post-operative model of pain in rats would create significant non-opioid dependent analgesia.

Methods

4.1 ADMSC growth and Secretome Production

Adipose tissue was acquired from an HREC approved procurement process under ethics approval number 2014-01-006. Adipose tissue was digested with collagenase as per (41), washed, and frozen as a

stromal vascular fraction (SVF) in liquid nitrogen.

AdMSCs were thawed and isolated via plastic adherence in tissue culture flasks. AdMSC identification was performed as per ISCT guidelines (42) cell surface characterisation via Flow Cytometry. Cells were confirmed to be positive for CD90 FITC, CD73 FITC, CD105 PE, CD166 PE and negative for HLA-DR FITC, CD45 FITC, CD31 FITC, CD106 PE and CD271 PE (Thermo Fisher, U.S.A).

Culturing of AdMSCs was performed under standard cell culture conditions (37°C, 5% CO_2) in Alpha Modified Eagle Medium (α-MEM; Lonza, U.S.A) supplemented with 10% human platelet lysate (PLT Gold, Mill Creek Life Sciences, U.S.A). AdMSCs were expanded to passage 6 in a multi-layer cell factory (10CF, Nunc, Thermo Fisher, U.S.A) and cryopreserved in CryoStor CS10 (Bio Life Solutions, U.S.A). The MSC-S was collected, centrifuged at 4000g to remove cell debris, and a portion frozen at -80°C to produce the low dose MSC-S group. The remaining portion was concentrated 5x using a 3kDa Tangential Flow Filtration (TFF) (Sartorius, Germany) to produce the high dose group before freezing at -80°C.

4.2 Study Design

The rat incision induced post-operative pain study was conducted by MD Biosciences Ltd (Weizmann Science Park Ness Ziona, Israel).

Four experimental groups were tested in the model (n=10 per group: Group 1 - Vehicle control consisting of α-MEM; Group 2 - Positive control, consisting of morphine (10 mg/kg) administered intraperitoneally 30 minutes post-surgery; Group 3 - low dose MSC-S; and Group 4 - high dose MSC-S. The study design is outlined in Figure 1.

4.3 Animals

This study was performed following approval of an application form submitted to the Committee for Ethical Conduct in the Care and Use of Laboratory Animals by MD Biosciences Ltd stating that the study complies with the rules and regulations of the ethics committee from MD Biosciences.

A total of 40 male Sprague Dawley (SD) rats weighing 180 to 200g (Envigo RMS (Israel), Ltd) were used for this study. Animals were acclimatised for at least five days and provided ad libitum with a commercial, sterile rodent diet and free access to drinking water. Environmental conditions were maintained at 17-23°C with a relative humidity of 30-70%, a 12:12 hour light: dark cycle and 15-30 air changes/h in the study room. Animals were randomly assigned to experimental groups. Each dosing group was kept in a separate cage to avoid cross-contamination, which can occur through the consumption of faecal matter. At the end of the study, animals were euthanised by sodium pentobarbital.

4.4. Induction of Post-Operative Pain

All animals were anesthetised by ketamine/xylazine solution. Under anaesthesia, a 1 cm longitudinal incision over the plantar surface of the right hind paw was performed, and the plantaris muscle was incised longitudinally. Following the surgery, the incision was closed with two stitches.

4.5 Treatment Administration

At 60 min post-surgery, 1ml of either vehicle, low dose MSC-S or high dose MSC-S were applied topically on a soaked gauze pad placed on top of the incision for 15 min. Morphine (10 mg/kg; Group 2) was administered intraperitoneally 30 min post-surgery.

4.6 Behavioural Testing

Von Frey filament test for mechanical induced hyperalgesia and allodynia:

Von Frey fibres were used to measure mechanical hyperalgesia and allodynia. Each rat was placed in an enclosure and positioned on a metal mesh surface but allowed to move freely. The test began after the cessation of exploratory behaviour. Fibres used ranged from 1.65 to 6.65 in size and 0.008 to 300 g force applied.

Hotplate test for assessing thermal hyperalgesia:

Thermal hyperalgesia was tested using a hotplate apparatus. Animals were placed on a hotplate apparatus (57±1ºC), and the time until a first response was recorded for both legs. The maximal time of testing was set at 12 s.

4.7 Multiplex Protein analysis

The high dose MSC-S was analysed using Custom 7 plex ProcartaPlex Multiplex Immunoassay, Cat # PPX-07-MXNKTH2, with analytes, HGF, IL-6, MCP-1, SDF-1a, TIMP-1, TNF-R1 and VEGF-a (Thermo Fisher, Australia) and custom six-plex with analytes IFN-g, IL-6, IL-8, MCP-1, TNF-a and VEGF-a. ProcartaPlex is a bead-based assay employing the sandwich ELISA technique and is analysed via the MAGPIX 200 (Luminex U.S.A).

4.8 Statistical Analysis

Statistical analysis of thermal and mechanical withdrawal thresholds was undertaken using a repeated measures 2-way analysis of variance (ANOVA) with the Geiser-Greenhouse correction utilising Dunnett's Multiple comparison test with individual variances computed for each comparison. Data are presented as Mean +/- SD.

Results

5.1.1 MSC-S creates dose-dependent alleviation of thermal post-operative pain in the rodent model

The POP model caused significant thermal hypernociception at 1.5 hours, as demonstrated by the vehicle control group where baseline measurements are significantly higher than 1.5 hours ($p<0.0001$) and 3 hours ($p<0.05$), which resolved by 5 hours (Figure 2). Treatment with all interventions caused significant attenuation of the hypernociception at 1.5 hours with high dose MSC S and morphine sustaining analgesia until 3 hours post-surgery (Figure 2). Hotplate measurements indicate a highly significant effect from the high dose MSC-S on thermal nociceptive thresholds at 1.5 hours ($p<0.0001$) and 3 hours post-surgery ($p<0.001$) when compared to Vehicle at the corresponding timepoint. A similar result was observed following morphine administration at 10 mg/kg (p-values of <0.001 and $p<0.04$ at 1.5 and 3 hours, respectively). However, treatment with high dose MSC-S resulted in significantly higher thermal nociceptive thresholds than morphine at 3 hours post-surgery ($p<0.04$). Significantly higher withdrawal thresholds were recorded in animals at 1.5 hours post-surgery in the low dose MSC-S group when compared to Vehicle ($p<0.001$).

5.1.2 MSC-S failed to alleviate post-operative mechanical allodynia in the rodent model

The POP model induced significant ($p<0.0001$) mechanical allodynia from 1.5 hours that was maintained throughout the observation window. Only morphine significantly attenuated this mechanical allodynia at 1.5 hours post-surgery ($p<0.03$).

Whilst rats that received morphine displayed significantly less allodynia than vehicle control mice at 1.5 hours post-surgery, this effect was not long-lasting (Figure 3). There were no other statistically significant differences in withdrawal threshold at any timepoint. No recovery to uninjured baseline withdrawal thresholds was observed at the 5-hour timepoint.

5.1.3 The post-operative rodent model does not impact animal body weight

Bodyweight measurements (Figure 4) were taken 1 hour prior to surgery. Body weights were not significantly different between groups as analysed using ordinary 2-way ANOVA using uncorrected Fishers LSD test. Bodyweight measurements indicate no variation between test groups indicating maintenance of animal welfare prior to the model being conducted.

5.1.4 Multiplex protein analysis of MSC-S reveal the complex bioactives present

The complex bioactives secreted by MSC that hypothesised to produce the physiological actions of MSC have been explored previously (43) and (38,44). This underlies the complex nature of the physiological activities of MSCs. A multiplex protein analysis was performed to provide information on the nature of the complex mixture of MSC-S utilised here. Our MSC-S contained detectable amounts of bioactive proteins, including IL-6, IL-8, MCP-1, VEGF-A, HGF, SDF-1a, TNF-R1 and TIMP-1 (figure 5).

Discussion

This study demonstrates that the MSC-S, when administered topically to rats following a hind paw incision, significantly affects thermal withdrawal latencies and, hence, hyperalgesia. Importantly, these antinociceptive actions were equivalent to 10 mg/kg morphine. To our knowledge, this is the first report of the pain-relieving effects of the MSC-S on post-operative pain.

Post-operative pain arises as a result of an inflammatory reaction caused by post-surgical trauma. During the normal injury process, an inflammatory response involving increased proinflammatory cytokine release at injury sites to promote immunoregulation and immune cell recruitment occurs (42). Although proinflammatory cytokine release can be beneficial for wound healing (42), it can contribute to the pathogenesis of pain as TNF-α and IL-1 cause rapid activity in C fibre and A-Delta nociceptors reducing mechanical activation thresholds (45). It has been demonstrated that IL-1 exposure to dorsal root ganglion induces spontaneous acute hyperexcitability of peripheral nociceptors and heat-induced hyperalgesia through sensitisation of the capsaicin and heat-sensitive cation channel TRPV1 receptors (45). The behavioural effects of this process resulting from the post-operative pain model were observed in this study, with heat-induced hyperalgesia being evident in Vehicle control animals. Our results illustrate a significant effect of topical administration of the high dose MSC-S in prolonging thermal latencies. However, no effect was observed in mechanical allodynia assessed by Von Frey testing. This is an intriguingly specific effect of the MSC-S and suggests the complex bioactives in the MSC-S either directly preferentially block the hyperexcitability in discrete thermal sensitive afferents, or reduces the generation of the endogenous factors that create the thermal rather than the mechanical sensitising agents. The complexity of this redundancy and parallel signalling pathways is exemplified in the recent pain interactome published by Science Signalling (46). Future studies examining these specific receptor and ligand effectors are warranted to identify which and how.

The MSC-S contains a milieu of chemokines, cytokines, growth factors and extracellular vesicles that promote angiogenesis and have anti-inflammatory properties (Figure 5) through T-cell suppression and immunoregulation (17,21,25,28,38). The potential therapeutic benefits of the MSC-S are well reported. However, given the duration of this study and the rapid effect of the MSC-S on thermal latencies, T cell suppression may not be the main mechanism of action due to the immediate response with a topical application. We postulate that molecules such as IL-1Ra, HGF, and TIMP-1 contained within the MSC-S may be responsible for the effects seen. IL-1Ra has also been shown to combat IL-1β induced hyperexcitability of peripheral nociceptors responsible for thermal hyperalgesia development (47–49). HGF and TIMP-1 were present in high MSC-S (Figure 5) levels and have been demonstrated to exert

analgesic and immunoregulatory effects (50,51). HGF may inhibit pain mediating genes in sensory neurons via interaction with c-MET receptors causing acute anti-hyperalgesic responses driven by the MSC-S (50). TIMP-1 has been demonstrated to attenuate inflammatory pain through matrix metalloproteinase inhibition and receptor-mediated cell signalling (51). IL-1Ra levels in the MSC-S were not measured in this study. Future studies will include an analysis of IL-1Ra levels in the MSC-S and blocking experiments to further investigate the role of key cytokines in pain. The upregulation of IL-1Ra secretion from MSCs under inflammatory stimulation has implications in pain-specific pathways and plays an important role in pain reduction and regulation (47–49).

Conclusions

The present study demonstrated that the MSC-S significantly improved thermal nociceptive thresholds when administered topically in a rat post-operative pain model.

Further investigation is required to elucidate the mechanism of action of the MSC-S. Specifically studies using cytokine depleted MSC-S and further immunological analysis through mRNA analysis at the site of injury would be beneficial to elucidate the effects of the MSC-S on hypernociception. A dynamic plantar aesthesiometer could be used in future studies due to increased sensitivity and repeatability compared to manual Von Frey fibres for the assessment of mechanical allodynia. The MSC-S has potential as a non-opioid based therapy for the treatment of acute post-operative pain.

Abbreviations

MSC – Mesenchymal Stem Cell

MSC-S – Mesenchymal Stem Cell Secretome

SD – Sprague Dawley

ANOVA – Analysis Of Variance

AdMSC – adipose-derived mesenchymal stem cell

SVF – Stromal Vascular Fraction

αMEM – alpha Modified Eagle Medium

PLT – Platelet lysate

TFF – Tangential Flow Filtration

DRG – Dorsal Root Ganglion

IP – Intraperitoneally

Declarations

Ethics approval and consent to participate

Adipose tissue was obtained with informed consent under ethics approved program, approval number, 2014-01-006. The animal study was performed following approval of an application form submitted to the Committee for Ethical Conduct in the Care and Use of Laboratory Animals by MD Biosciences Ltd and stated that the study complies with the rules and regulations of the AEC.

Consent for publication

Not Applicable

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

Graham Vesey is a shareholder and executive director of Regeneus Ltd.

Funding

Funding was provided from Regeneus Ltd. MRH is the recipient of an ARC Future Fellowship (FT180100565).

Authors' contributions

CM interpreted data and wrote the manuscript. GV, SB and FM revised the work and manuscript. MH contributed to the manuscript and data analysis.

Acknowledgements

We acknowledge the support of MD Biosciences Ltd (Weizmann Science Park Ness Ziona, Israel), who conducted the animal studies under study number MD-3-1-700-2047

References

1. Gan TJ. Poorly controlled postoperative pain: Prevalence, consequences, and prevention. J Pain Res. 2017;10:2287–98.

2. Mwaka G, Thikra S, Mung'ayi V. The prevalence of postoperative pain in the first 48 hours following day surgery at a tertiary hospital in Nairobi. Afr Health Sci. 2013;13(3):768–76.

3. Johansen A, Romundstad L, Nielsen CS, Schirmer H, Stubhaug A. Persistent postsurgical pain in a general population: Prevalence and predictors in the Tromsø study. Pain [Internet]. 2012;153(7):1390–6. Available from: http://dx.doi.org/10.1016/j.pain.2012.02.018

4. Kehlet H, Jensen TS, Woolf CJ. Persistent postsurgical pain: risk factors and prevention. Lancet. 2006;367(9522):1618–25.

5. Wu CL, Raja SN. Treatment of acute postoperative pain. Lancet [Internet]. 2011;377(9784):2215–25. Available from: http://dx.doi.org/10.1016/S0140-6736(11)60245-6

6. Dahl JB, Mathiesen O, Kehlet H. An expert opinion on postoperative pain management, with special reference to new developments. Expert Opin Pharmacother. 2010;11(15):2459–70.

7. Gupta A, Kaur K, Sharma S, Goyal S, Arora S, Murthy RSR. Clinical aspects of acute post-operative pain management & its assessment [Internet]. Vol. 1, Journal of Advanced Pharmaceutical Technology and Research. Wolters Kluwer – Medknow Publications; 2010 [cited 2020 Nov 24]. p. 97–108. Available from: /pmc/articles/PMC3255434/?report=abstract

8. C E, TD J, D K, VG L, B S, R H, et al. Adverse events associated with medium- and long-term use of opioids for chronic non-cancer pain: an overview of Cochrane Reviews (Review). 2017;

9. Karlsen APH, Wetterslev M, Hansen SE, Hansen MS, Mathiesen O, Dahl JB. Postoperative pain treatment after total knee arthroplasty: A systematic review. Vol. 12, PLoS ONE. 2017. 1–53 p.

10. Moore RA, Derry S, Aldington D, Wiffen PJ. Single-dose oral analgesics for acute postoperative pain in adults - an overview of Cochrane reviews. Cochrane Database Syst Rev. 2014;2014(11).

11. Zuk PA, Zhu M, Ashjian P, De Ugarte DA, Huang JI, Mizuno H, et al. Human Adipose Tissue Is a Source of Multipotent Stem Cells □ D. Mol Biol Cell [Internet]. 2002 [cited 2019 Feb 12];13:4279–95. Available from: www.molbiolcell.org.

12. Nho B, Lee J, Lee J, Ryang Ko K, Joong Lee S, Kim S. Effective control of neuropathic pain by transient expression of hepatocyte growth factor in a mouse chronic constriction injury model. [cited 2018 Sep 6]; Available from: www.fasebj.org

13. Klass M, Gavrikov V, Drury D, Stewart B, Hunter S, Denson DD, et al. Intravenous mononuclear marrow cells reverse neuropathic pain from experimental mononeuropathy. Anesth Analg. 2007;104(4):944–8.

14. Guo W, Wang H, Zou S, Gu M, Watanabe M, Wei F, et al. Bone marrow stromal cells produce long-term pain relief in rat models of persistent pain. Stem Cells [Internet]. 2011 Aug [cited 2019 Feb 22];29(8):1294–303. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21630378

15. Totey S, Pal R, Gopinath C, Rao NM, Banerjee P, Krishnamoorthy V, et al. Functional recovery after transplantation of bone marrow-derived human mesenchymal stromal cells in a rat model of spinal cord injury. 2010 [cited 2019 Mar 21]; Available from: https://ac-els-cdn-com.wwwproxy1.library.unsw.edu.au/S146532491070445X/1-s2.0-S146532491070445X-main.pdf?_tid=3e36bce2-c83b-40b3-a8b7-bf7f258fe0c3&acdnat=1553126070_977d62b073d15f8982219c52b6b768f1

16. Bennett GJ, Xie Y-K. A peripheral mononeuropathy in rat that produces disorders of pain sensation like those seen in man [Internet]. Vol. 33, Pain. 1988 [cited 2019 Feb 22]. Available from: https://ac-els-cdn-com.wwwproxy1.library.unsw.edu.au/0304395988902096/1-s2.0-0304395988902096-main.pdf?_tid=ee55f7de-b043-4116-bd93-bc3d91a881c0&acdnat=1550812109_06680d18ab6d161bcd3776507a7d5eee

17. Mert T, Kurt AH, Altun İ, Celik A, Baran F, Gunay I. Pulsed magnetic field enhances therapeutic efficiency of mesenchymal stem cells in chronic neuropathic pain model. Bioelectromagnetics [Internet]. 2017 May 1 [cited 2019 Feb 22];38(4):255–64. Available from: http://doi.wiley.com/10.1002/bem.22038

18. Waterman RS, Betancourt AM. Treating Chronic Pain with Mesenchymal Stem Cells: A Therapeutic Approach Worthy of Continued Investigation. J Stem Cell Res Ther [Internet]. 2011 Oct 21 [cited 2018 Sep 5];01(S2):1–5. Available from: https://www.omicsonline.org/treating-chronic-pain-with-mesenchymal-stem-cells-a-therapeutic-approach-worthy-of-continued-investigation-2157-7633.S2-001.php?aid=2144

19. Xie F, Yue Y, Guan Y, Wang Y. Stem Cells for the Treatment of Neuropathic Pain [Internet]. [cited 2019 Feb 22]. Available from: http://www.japmnet.com/uploadfile/2017/0121/20170121044012254.full.pdf

20. Musolino PL, Coronel MF, Hökfelt T, Villar MJ. Bone marrow stromal cells induce changes in pain behavior after sciatic nerve constriction. Neurosci Lett [Internet]. 2007 [cited 2019 Mar 21];418:97–101. Available from: https://ac-els-cdn-com.wwwproxy1.library.unsw.edu.au/S0304394007002704/1-s2.0-S0304394007002704-main.pdf?_tid=d01361e3-f128-46f9-b597-5b56722ffb71&acdnat=1553124775_3c056555fc965f3eab07c5b257fab1bf

21. Chen C, Chen F, Yao C, Shu S, Feng J, Hu X, et al. Intrathecal Injection of Human Umbilical Cord-Derived Mesenchymal Stem Cells Ameliorates Neuropathic Pain in Rats. Neurochem Res [Internet]. 2016;41(12):3250–60. Available from: http://dx.doi.org/10.1007/s11064-016-2051-5

22. Urdzíková L, Jendelová P, Rina Glogarová K, Burian M, Hájek M, Syková E. Transplantation of Bone Marrow Stem Cells as well as Mobilization by Granulocyte-Colony Stimulating Factor Promotes

Recovery after Spinal Cord Injury in Rats. J Neurotrauma [Internet]. 2006 [cited 2019 Mar 21];23(9). Available from: https://search-proquest-com.wwwproxy1.library.unsw.edu.au/docview/204523852?rfr_id=info%3Axri%2Fsid%3Aprimo

23. Sun Y, Zhang D, Li H, Long R, Sun Q. Intrathecal administration of human bone marrow mesenchymal stem cells genetically modified with human proenkephalin gene decrease nociceptive pain in neuropathic rats. Mol Pain [Internet]. 2017 [cited 2018 Sep 17];13:1744806917701445. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28326940

24. Schäfer S, Berger J V, Deumens R, Goursaud S, Hanisch U-K, Hermans E. Influence of intrathecal delivery of bone marrow-derived mesenchymal stem cells on spinal inflammation and pain hypersensitivity in a rat model of peripheral nerve injury [Internet]. 2014 [cited 2019 Mar 21]. Available from: http://www.jneuroinflammation.com/content/11/1/157

25. Chiang C-Y, Liu S-A, Sheu M-L, Chen F-C, Chen C-J, Su H-L, et al. Feasibility of Human Amniotic Fluid Derived Stem Cells in Alleviation of Neuropathic Pain in Chronic Constrictive Injury Nerve Model. Costigan M, editor. PLoS One [Internet]. 2016 Jul 21 [cited 2019 Feb 22];11(7):e0159482. Available from: https://dx.plos.org/10.1371/journal.pone.0159482

26. Hosseini M, Yousefifard M, Aziznejad H, Nasirinezhad F. The Effect of Bone MarroweDerived Mesenchymal Stem Cell Transplantation on Allodynia and Hyperalgesia in Neuropathic Animals: A Systematic Review with Meta-Analysis. Biol Blood Marrow Transplant [Internet]. 2015 [cited 2019 Feb 22];21:1537–44. Available from: http://dx.doi.org/10.1016/j.bbmt.2015.05.008

27. Gama KB, Santos DS, Evangelista AF, Silva DN, de Alcântara AC, dos Santos RR, et al. Conditioned Medium of Bone Marrow-Derived Mesenchymal Stromal Cells as a Therapeutic Approach to Neuropathic Pain: A Preclinical Evaluation. Stem Cells Int [Internet]. 2018 Jan 30 [cited 2018 Sep 5];2018:1–12. Available from: https://www.hindawi.com/journals/sci/2018/8179013/

28. Maione S, Rossi F, de Novellis V, Galderisi U, Luongo L, Giordano C, et al. Long-Lasting Effects of Human Mesenchymal Stem Cell Systemic Administration on Pain-Like Behaviors, Cellular, and Biomolecular Modifications in Neuropathic Mice. Front Integr Neurosci. 2011;5(December):1–10.

29. Huh Y, Ji R-R, Chen G. Neuroinflammation, Bone Marrow Stem Cells, and Chronic Pain. Front Immunol [Internet]. 2017 Aug 21 [cited 2018 Sep 5];8(August):1014. Available from: http://journal.frontiersin.org/article/10.3389/fimmu.2017.01014/full

30. Kishk NA, Gabr H, Hamdy S, Afifi L, Abokresha N, Mahmoud H, et al. Case Control Series of Intrathecal Autologous Bone Marrow Mesenchymal Stem Cell Therapy for Chronic Spinal Cord Injury. [cited 2019 Mar 21]; Available from: http://nnr.sagepub.com

31. Franchi S, Valsecchi AE, Borsani E, Procacci P, Ferrari D, Zaffa C, et al. Intravenous neural stem cells abolish nociceptive hypersensitivity and trigger nerve regeneration in experimental neuropathy. Pain

[Internet]. 2012 [cited 2019 Feb 22];153:850–61. Available from: https://ac-els-cdn-com.wwwproxy1.library.unsw.edu.au/S0304395912000097/1-s2.0-S0304395912000097-main.pdf?_tid=27f4fe4b-8e04-4b2e-8eed-fe40beb34421&acdnat=1550808909_a4818b330540db0464d10cf427b47612

32. Wei F, Guo W, Zou S, Ren K, Dubner R. Supraspinal glial-neuronal interactions contribute to descending pain facilitation. J Neurosci [Internet]. 2008 Oct 15 [cited 2019 Mar 21];28(42):10482–95. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18923025

33. Parr AM, Tator CH, Keating A. Bone marrow-derived mesenchymal stromal cells for the repair of central nervous system injury. Bone Marrow Transplant [Internet]. 2007 [cited 2019 Mar 21];40:609–19. Available from: www.nature.com/bmt

34. Genc D, Zibandeh N, Yildiz Y, Aslan S, Demirtas F, Karaoz E, et al. Effects of Mesenchymal Stromal Cells on the Neuropathic Pain Induced by Chronic Constriction Injury in Rats. J Pain Reli [Internet]. 2017 Sep 25 [cited 2019 Feb 22];06(05):1–7. Available from: https://www.omicsonline.org/open-access/effects-of-mesenchymal-stromal-cells-on-the-neuropathic-pain-induced-by-chronic-constriction-injury-in-rats-2167-0846-1000302.php?aid=93917

35. Borsani E, Franchi S, Rossi A, Panerai AE, Maione S, Rodella LF. stem cell transplantation in neuropathic pain. 2011;1.

36. Klein S, Svendsen CN. Stem cells in the injured spinal cord: Reducing the pain and increasing the gain. Nat Neurosci. 2005;8(3):259–60.

37. Karaoz E, Ayça AE, Ae A, Ayhan S, Ayla AE, Sarıboyacı E, et al. Characterization of mesenchymal stem cells from rat bone marrow: ultrastructural properties, differentiation potential and immunophenotypic markers. [cited 2019 Feb 27]; Available from: https://link-springer-com.wwwproxy1.library.unsw.edu.au/content/pdf/10.1007/s00418-009-0629-6.pdf

38. Chen G, Park C-K, Xie R-G, Ji R-R. Intrathecal bone marrow stromal cells inhibit neuropathic pain via TGF-β secretion. J Clin Invest. 2015;

39. Song CH, Zhang EJ, Ko YK, Lee WH. Intrathecal Administration of Mesenchymal Stem Cells Reduces the Reactive Oxygen Species and Pain Behavior in Neuropathic Rats. Korean J Pain [Internet]. 2014 [cited 2019 Mar 21];27(3). Available from: http://dx.doi.org/10.3344/kjp.2014.27.3.239www.epain.org

40. Brini AT, Amodeo G, Ferreira LM, Milani A, Niada S, Moschetti G, et al. Therapeutic effect of human adipose-derived stem cells and their secretome in experimental diabetic pain. Sci Rep [Internet]. 2017 Dec 29 [cited 2019 Feb 22];7(1):9904. Available from: http://www.nature.com/articles/s41598-017-09487-5

41. Zuk PA, Zhu M, Mizuno H, Huang J, Futrell JW, Katz AJ, et al. Multilineage cells from human adipose tissue: Implications for cell-based therapies. In: Tissue Engineering. 2001. p. 211–28.

42. Galipeau J, Krampera M, Barrett J, Dazzi F, Deans RJ, DeBruijn J, et al. International Society for Cellular Therapy perspective on immune functional assays for mesenchymal stromal cells as potency release criterion for advanced phase clinical trials. Cytotherapy. 2015;

43. K.B. G, D.S. S, A.F. E, D.N. S, A.C. DA, R.R. DS, et al. Conditioned Medium of Bone Marrow-Derived Mesenchymal Stromal Cells as a Therapeutic Approach to Neuropathic Pain: A Preclinical Evaluation. Stem Cells Int [Internet]. 2018;2018:8179013. Available from: http://www.hindawi.com/journals/sci/contents/%0Ahttp://ovidsp.ovid.com/ovidweb.cgi?T=JS&PAGE=reference&D=emexb&NEWS=N&AN=620705485

44. Hofer HR, Tuan RS. Secreted trophic factors of mesenchymal stem cells support neurovascular and musculoskeletal therapies. Stem Cell Res Ther [Internet]. 2016 Dec 9 [cited 2017 Nov 8];7(1):131. Available from: http://stemcellres.biomedcentral.com/articles/10.1186/s13287-016-0394-0

45. Shubayev VI, Kato K, Myers RR. Cytokines in pain. Transl Pain Res From Mouse to Man. 2009;187–214.

46. Wangzhou A, Paige C, Neerukonda S V., Naik DK, Kume M, David ET, et al. A ligand-receptor interactome platform for discovery of pain mechanisms and therapeutic targets. Sci Signal. 2021;14(674):1–22.

47. Grace PM, Hutchinson MR, Maier SF, Watkins LR. Pathological pain and the neuroimmune interface. Nat Rev Immunol. 2014;

48. Hutchinson MR, Coats BD, Lewis SS, Zhang Y, Sprunger DB, Rezvani N, et al. Proinflammatory cytokines oppose opioid-induced acute and chronic analgesia. Brain Behav Immun. 2008;

49. Zhang J-M, An J. Cytokines, Inflammation, and Pain. Int Anesthesiol Clin. 2007;

50. Nho B, Lee J, Lee J, Ryang Ko K, Joong Lee S, Kim S. Effective control of neuropathic pain by transient expression of hepatocyte growth factor in a mouse chronic constriction injury model. Available from: www.fasebj.org

51. Knight BE, Kozlowski N, Havelin J, King T, Crocker SJ, Young EE, et al. TIMP-1 Attenuates the Development of Inflammatory Pain Through MMP-Dependent and Receptor-Mediated Cell Signaling Mechanisms. Front Mol Neurosci. 2019;12(September):1–16.

Figures

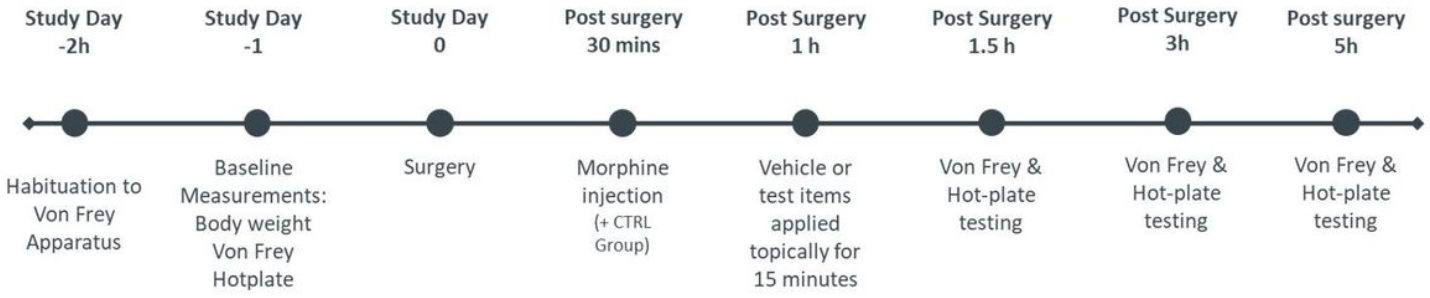

Figure 1

Study design

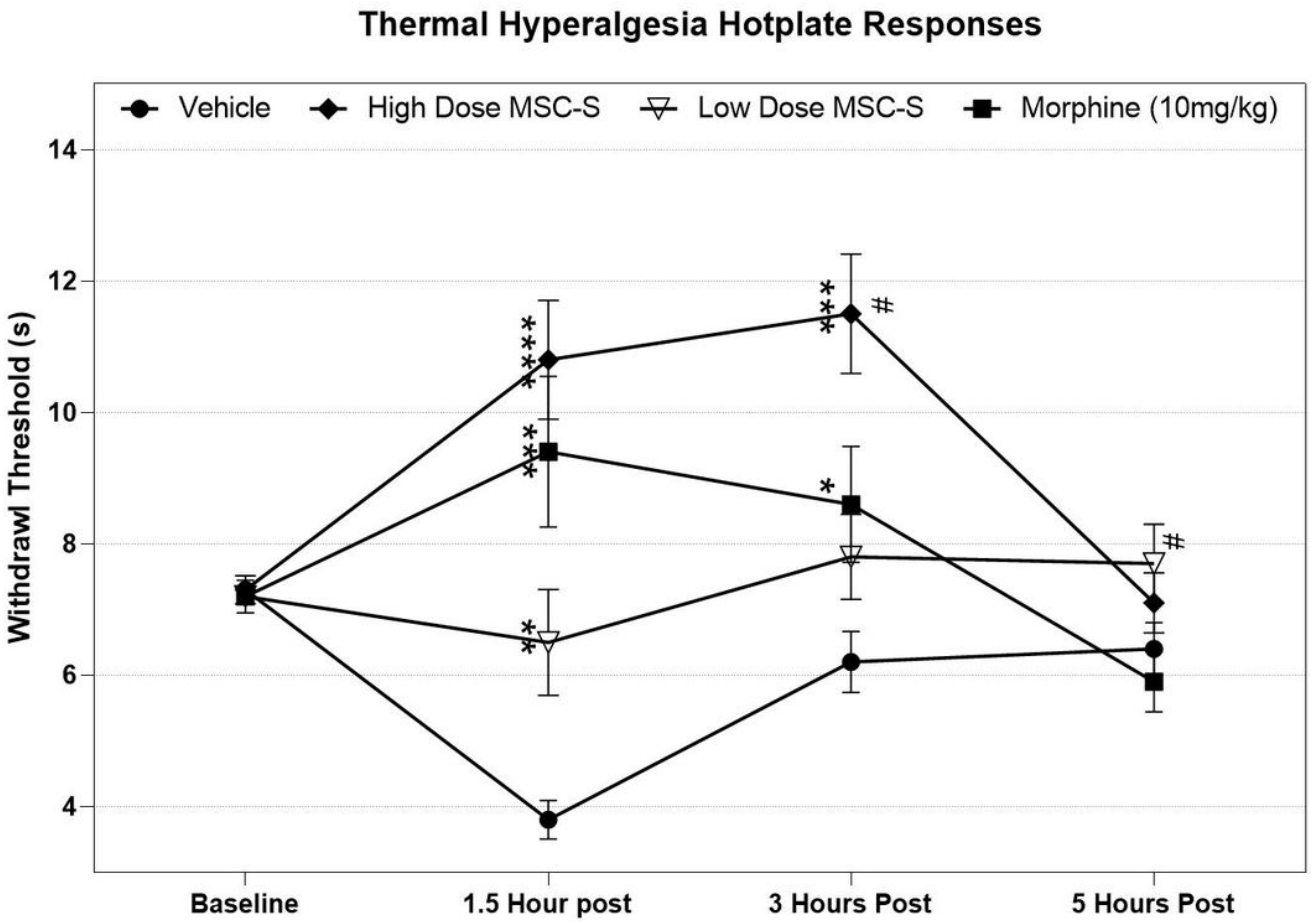

Figure 2

Thermal Hyperalgesia Hotplate Response, mean +/- SEM, n=10 per treatment group. Statistical analysis was undertaken using a repeated measures 2-way analysis of variance (ANOVA) with the Geiser-Greenhouse correction utilising Dunnett's Multiple comparison test with individual variances computed for each comparison. * $p<0.04$ significantly different from Vehicle at the same timepoint ** $p<0.01$ significantly different from Vehicle at the same timepoint *** $p<0.001$ significantly different from Vehicle at the same timepoint **** $p<0.0001$ significantly different from Vehicle at the same timepoint # $p<0.04$ Significantly different from Morphine at the same timepoint

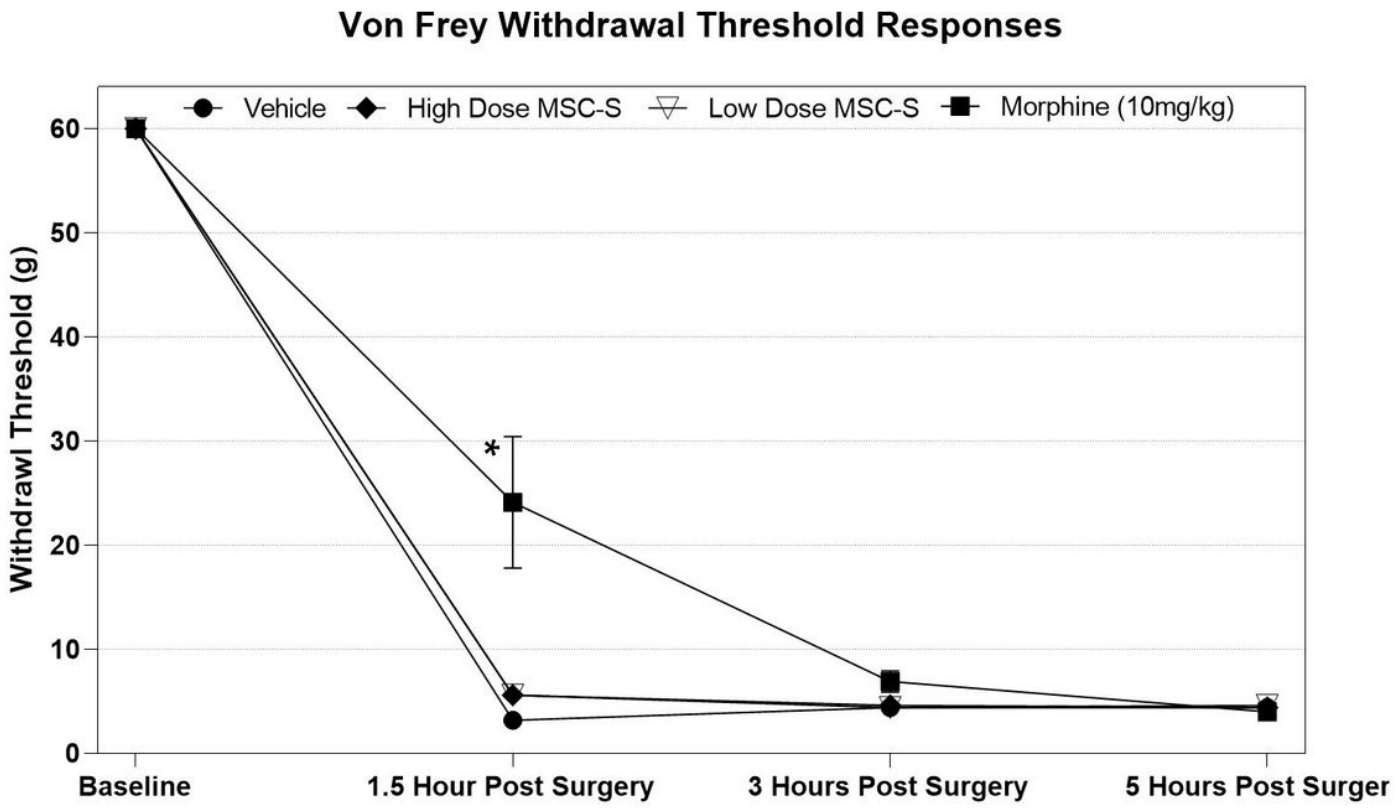

Figure 3

Von Frey Withdrawl Threshold responses (g), mean +/- SEM, n=10 per treatment group. Statistical analysis was undertaken using a repeated measures 2-way analysis of variance (ANOVA) with the Geiser-Greenhouse correction utilising Dunnett's Multiple comparison test with individual variances computed for each comparison. * $p<0.03$ significantly different from Vehicle at the same timepoint

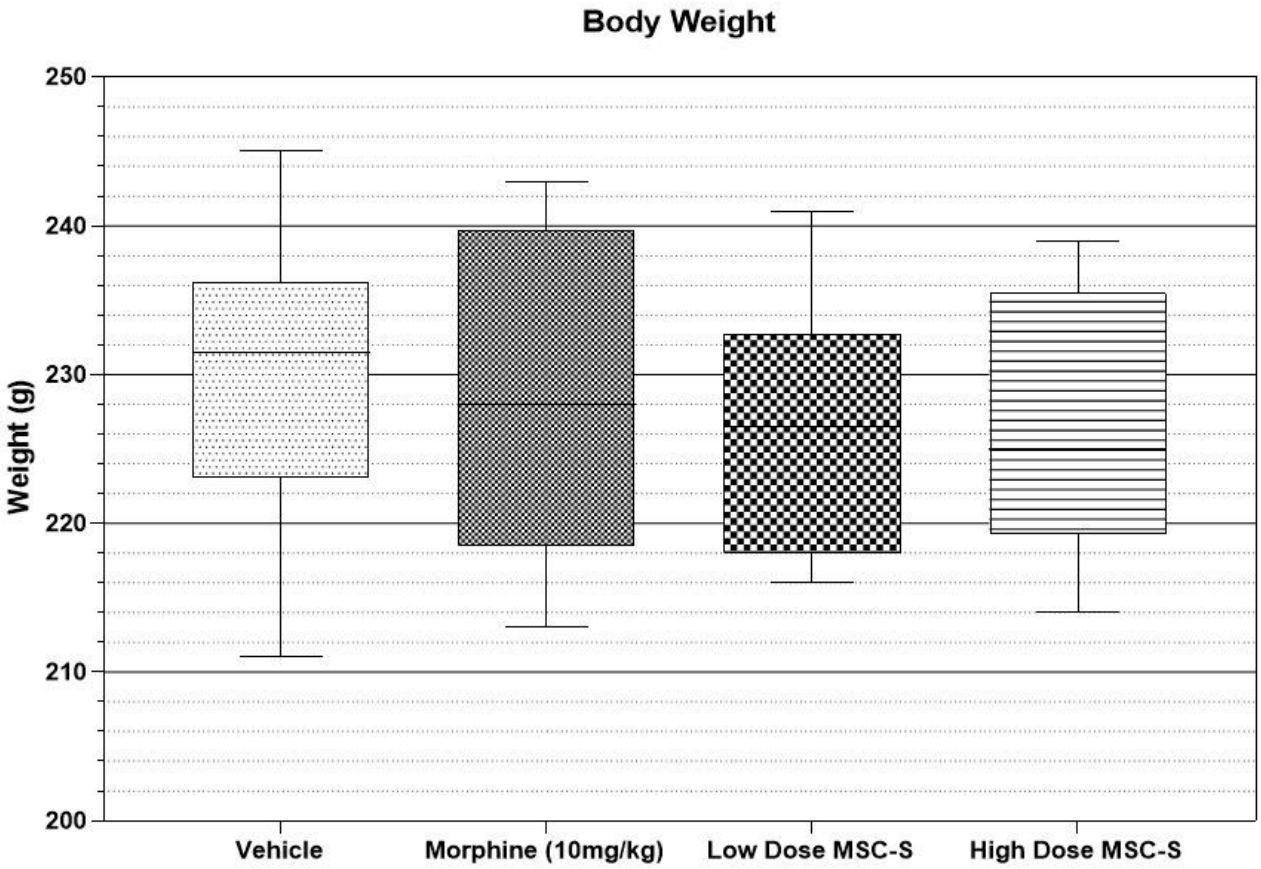

Figure 4

Body Weight (g), mean +/- SD n=10 per treatment group

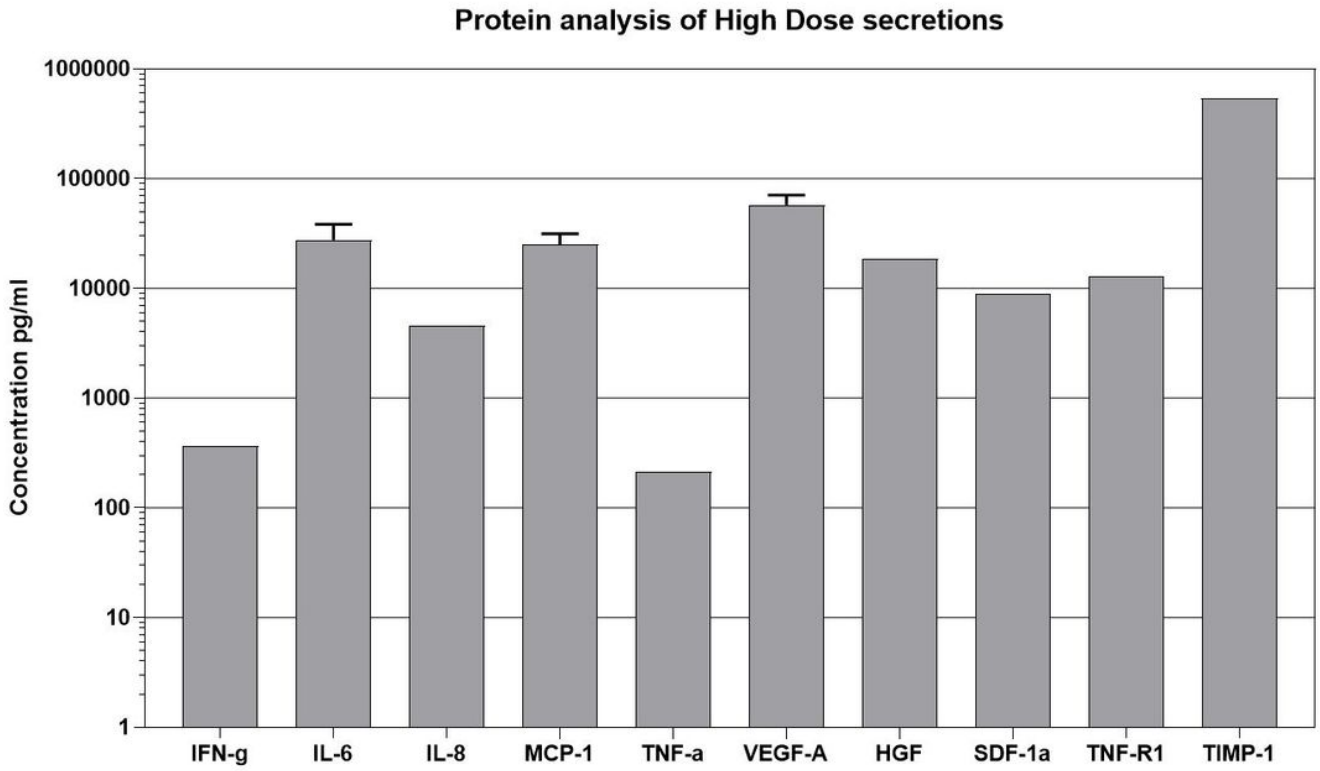

Figure 5

Protein analysis of High Dose MSC-S group, mean pg/ml +/- SD